

Supercooling along an Altitudinal Gradient in *Espeletia schultzii*, a Caulescent Giant Rosette Species

FERMIN RADA, GUILLERMO GOLDSTEIN, AURA AZOCAR AND FRESIA TORRES

Departamento de Biología, Facultad de Ciencias, Universidad de los Andes, Merida, Venezuela

Received 19 August 1986

ABSTRACT

Rada, F., Goldstein, G., Azocar, A. and Torres, F. 1987. Supercooling along an altitudinal gradient in *Espeletia schultzii*, a caulescent giant rosette species.—J. exp. Bot. 38: 491–497.

Tropical high Andes plants may be exposed to sub-zero temperatures any night of the year. These plants have to rely on mechanisms which protect them from these environmental conditions but at the same time allow their growth and development. Supercooling has been found to be the principal avoidance mechanism in leaves of the caulescent giant rosette genus *Espeletia* in the Andes. We report here the differences in supercooling capacity and cold injury in several *Espeletia schultzii* populations growing along an altitudinal gradient. The relationships between supercooling, water potential and leaf anatomy were also investigated. The supercooling capacity increased and injury temperature decreased from lower to higher elevation populations. These changes may be explained in terms of physiological, morphological and anatomical characteristics of the leaves.

Key words—*Espeletia schultzii*, supercooling, freezing avoidance mechanisms.

Correspondence to: Departamento de Biología, Facultad de Ciencias, Universidad de los Andes, Merida, Venezuela.

INTRODUCTION

Supercooling is known to be an effective mechanism against freezing injury in only a limited number of plant species (Larcher, 1982; George, Burke, Pellet, and Johnson, 1974; Kaku and Iwaya, 1982). Larcher (1971) and Levitt (1980) suggested that supercooling should be selected as a freezing resistance mechanism in regions where light frosts occur during periods of high metabolic and developmental activity. In some high tropical environments where these characteristics are present due to the possibility of below zero temperatures during any night of the year, however, frost tolerance as opposed to its avoidance has been observed. This is the case of the giant caulescent rosette species of tropical high mountains in Africa, where freezing of the leaves occurs without damage (Beck, Senser, Scheibe, Steiger, and Pontgrantz, 1982; Beck, Schulze, Senser, and Scheibe, 1984). In the high Andes, leaf tissues of *Espeletia* species supercool to relatively low temperatures, avoiding extracellular freezing of leaf tissues (Rada, Goldstein, Azocar, and Meinzer, 1985; Goldstein, Rada, and Azocar, 1985). The species-specific supercooling points coincide with the temperature at which significant tissue damage was observed, indicating that these species are not capable of tolerating extracellular ice formation.

Espeletia schultzii Wedd (Compositae) is a giant caulescent rosette species occurring along an elevational gradient from 2 600 m to 4 200 m in the Venezuelan Andes. These plants

consist of a stem covered by marcescent leaves which maintain the stem temperature above 0 °C, and an apical rosette of densely pubescent leaves. The apical bud is insulated by layers of expanding and mature leaves, which at night bend inward by means of nyctinastic movements (Smith, 1974). Temperatures below 0 °C were never observed in these protected stem, pith and bud tissues (Smith, 1974, 1979; Goldstein and Meinzer, 1983; Rada, 1983). A series of morphological characteristics of *Espeletia schultzii* change along the altitudinal gradient: total plant height as elevation increases, pith volume per unit leaf area increases with elevation resulting in a larger water storage capacity at higher elevations (Goldstein, Meinzer, and Monasterio, 1984; Meinzer and Goldstein, 1984), smaller and more erect leaves with a thicker pubescent layer occur as elevation increases (Meinzer, Goldstein, and Rundel, 1985).

The purpose of the present study was to determine the supercooling capacity and freezing injury in *Espeletia schultzii* populations growing under different temperature regimes. The relationships between supercooling, leaf water potential components and leaf anatomy are also discussed.

MATERIALS AND METHODS

Five *Espeletia schultzii* Wedd populations were studied along an altitudinal gradient (Table 1) in the Venezuelan Andes (approximate coordinates lat. 8° 52' N, long. 70° 45' W). The lowest population, Santo Domingo, is found at an elevation of 2 600 m where the plants occur in abandoned fields. The remaining four populations exist as components of the natural paramo (tropical alpine) vegetation: Los Plantios (3 100 m), Mucubaji (3 550 m), Trasandina (3 800 m) and Piedras Blancas (4 200 m). This 2 600–4 200 m altitudinal gradient corresponds to a climatic gradient of decreasing temperature with 3 200 m marking a rapid increase in the frequency of night frosts (Monasterio and Reyes, 1980). Plants were excavated from each site with roots and soil intact, transported to the laboratory and placed in a growth chamber with controlled temperature and irradiance simulating field conditions.

Cold injury

The refined TTC method described by Steponkus and Lanphear (1967) was used to determine tissue injury. Tissue samples from mature leaves were cut and immediately placed in sealed tubes and immersed in an alcohol refrigerated bath (Grant Instruments Ltd.). Temperature was lowered from 10 °C to –30 °C at a rate of 10 °C h⁻¹. This cooling rate was selected because it was similar to the maximum temperature change at the end of the daytime period observed in the field. Three replicas of each sample were taken from the bath at 5 °C intervals and incubated at 6 °C for 8 h. After this incubation period, the TTC solution was applied and left for 15 h. An ethanol extract was then made and the absorbance at 530 nm measured (Spectronic 20, Bausch and Lomb). Freezing injury was defined as the amount of TTC reduced by the samples which resulted in 50% absorbance of the amount of TTC reduced by the unfrozen reference sample at 10 °C. This temperature was selected as 100% absorbance because it was the temperature at which the plants were maintained under control conditions before measurements were made. Steponkus and Lanphear (1967) have used 5 °C as 100% absorbance because they have used tissue samples from plants that were acclimated at this temperature. The slight decrease in absorbance between 10 °C and 0 °C may have been caused by sampling errors although this decrease was very small compared to the one observed below 0 °C.

Thermal analysis

In order to determine the temperature at which leaf tissue freezing occurred, pieces 3.0 cm long and 1.0 cm wide were cut and immediately enclosed in small tightly sealed tubes to avoid changes in tissue water content. Copper–constantan thermocouples (36 gauge) were inserted in the leaf sample and temperature changes were continuously monitored with a strip chart recorder fitted with an electronic 0 °C reference. Prior to immersion in a refrigerated alcohol bath the tubes were enclosed in an aluminium cylinder which acted as a heat sink and provided temperature stabilization during the cooling process (Quamme, Stushnoff, and Weiser, 1972). The temperature of the bath was lowered from 10 °C to –30 °C at a rate of 10 °C h⁻¹. The temperature at which freezing of the leaf tissue occurred was determined by the marked increase in leaf temperature due to the exothermic process of ice formation.

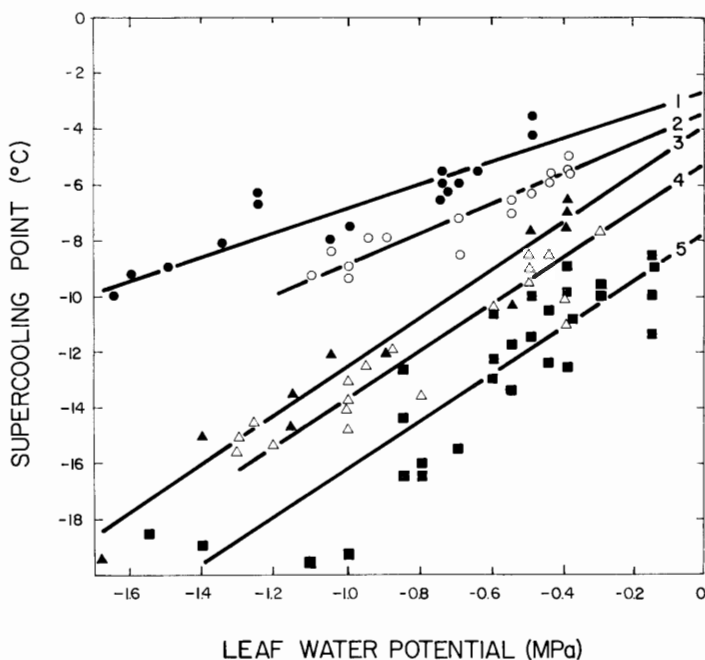


FIG. 1. Relationship between leaf water potential and supercooling point for five *Espeletia schultzii* populations: Santo Domingo, 2 600 m (●); Los Plantios, 3 100 m (○); Mucubaji, 3 550 m (▲); Trasandina, 3 800 m (△) and Piedras Blancas, 4 200 m (■). Numbers on regression lines for each population refer to Table 1.

Leaf water potential (Ψ_L) and supercooling capacity

The effect of Ψ_L on supercooling was investigated by determining the supercooling capacity of leaves of different Ψ_L . Leaves were cut from the rosette and allowed to transpire freely for different lengths of time in order to obtain a wide range of Ψ_L values. At each time interval a small section of the leaf was placed in the bath tubes and the thermal analysis procedure carried out. The rest of the leaf was immediately put in the pressure chamber for Ψ_L determination.

Relative apoplastic water content

Relative apoplastic water content was obtained in a manner similar to the one suggested by Richter, Duhme, Glatzel, Hinckley, and Karlic (1980). Rehydrated leaves were submerged in liquid nitrogen and then slowly dehydrated while Ψ_L was measured at regular intervals. From the relative water loss needed to make $\Psi_L = \Psi_{\pi}^{100}$ we calculated the relative apoplastic water content.

RESULTS

Figure 1 presents the relationship between supercooling and Ψ_L for the five *Espeletia schultzii* populations. Supercooling capacity increased linearly with decreasing leaf water potentials, with plants from higher elevations being more sensitive to changes in Ψ_L . At a given leaf water potential the supercooling capacity increased from the lowest elevation (Santo Domingo, 2 600 m) to the highest one (Piedras Blancas, 4 200 m). The slopes (b) of the relationship for the two lower elevations, Santo Domingo ($b = 0.40$) and Los Plantios ($b = 0.54$), were lower relative to the other three higher elevation sites: Mucubaji ($b = 0.82$), Trasandina ($b = 0.77$) and Piedras Blancas ($b = 0.85$) ($P < 0.05$, $Q(4, 82) = 7.75$; Sokal and Rohlf, 1981).

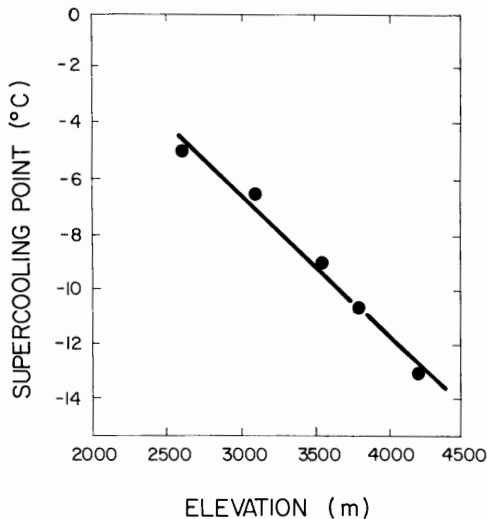


FIG. 2. Supercooling points as a function of elevation for five *Espeletia schultzii* populations. The supercooling temperatures were taken at $\Psi_L = -0.6$ MPa from Fig. 1. The regression equation is: supercooling = $7.824 + (-0.00495 \times \text{elevation})$

A strong linear relationship between elevation and supercooling capacity (Fig. 2) was also obtained for the five populations. For every 100 m change in elevation, the supercooling capacity increased approximately 0.5°C . The supercooling points in this figure were selected from Fig. 1 using a leaf water potential of -0.6 MPa, a value which generally occurs during the early morning hours when the lowest air temperatures are observed. This rate of change in supercooling with elevation is comparable to the rates of change of minimum air temperature with elevation. Such rates vary from place to place in tropical areas (Schubert and Medina, 1982) with the rate for the Venezuelan Andes being $-0.58^\circ\text{C}/100$ m (obtained from nine weather stations with at least nine years of continuous records). The rate of change in the supercooling capacity observed in *Espeletia schultzii* as well as the correlation coefficient of the regression ($r = 0.98$) can be even further improved if the Santo Domingo site is removed from the regression analysis. This population is found more than 500 m below the limit of night frosts (3 200 m) and, therefore, supercooling may not be important as a resistance mechanism against freezing. Omitting this population, a rate of change of $-0.57^\circ\text{C}/100$ m in the supercooling capacity is obtained, one which corresponds closely to the $-0.58^\circ\text{C}/100$ m value for minimum air temperature.

The temperature at which freezing injury occurred also showed a trend along the altitudinal gradient (Fig. 3). 50% tissue injury occurred at -7.4°C for Santo Domingo and at -13.3°C for Piedras Blancas, with temperatures within this range for the other populations.

The ability of a plant tissue to exist in a supercooled state depends on physical factors such as cell size and the intercellular spaces available for ice nucleation (Levitt, 1980). Epidermal and mesophyll cell size decreased with increasing altitude, as did the relative apoplastic water content, which is an indirect measure of intercellular volume (Table 1). Plants from the Santo Domingo site (2 600 m) had a relative apoplastic water content of more than 30% while plants from Piedras Blancas (4 200 m) had values less than 4%. Relative apoplastic water content values were comparable to those obtained with the pressure-volume method described by Tyree and Hammel (1972). With respect to cell size, both epidermal and mesophyll cells are much larger in the Santo Domingo plants, approximately $50\ \mu\text{m}$ and $30\ \mu\text{m}$, respectively, as

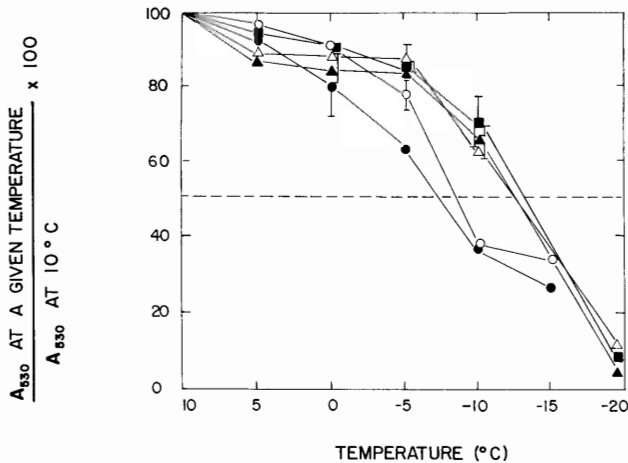


FIG. 3. Percent absorbances at 530 nm (A_{530}) as a function of leaf temperature for five *Espeletia schultzei* populations: Santo Domingo, 2600 m (●); Los Plantios, 3100 m (○); Mucubaji, 3550 m (▲); Trasandina, 3800 m (△); and Piedras Blancas, 4200 m (■). Dashed line indicates 50% injury.

compared to Piedras Blancas individuals where the epidermal cells are approximately $30\ \mu\text{m}$ and the mesophyll cells are close to $20\ \mu\text{m}$. There is a clear correlation between epidermal cell size and elevation ($r = 0.92$) and for mesophyll cell size and elevation ($r = 0.94$).

DISCUSSION

Supercooling appears to play an important role in the cold resistance mechanisms of Andean giant rosette plants. These results for *Espeletia schultzei* support those obtained for other *Espeletia* species where a very close relationship was found between freezing injury and supercooling capacity (Rada, 1983; Rada *et al.*, 1985; Goldstein *et al.*, 1985). Freezing after a marked supercooling, however, is far more likely to be fatal than the gradual freezing that occurs when there is no marked supercooling leading Levitt (1980) to consider supercooling an unlikely survival strategy. The relatively constant temperatures and moderate freezes of short duration which characterize high Andean environments, make it possible for *Espeletia schultzei* to utilize supercooling as an important resistance mechanism for the protection of the leaves from freezing.

The Santo Domingo and Los Plantios populations exhibit a relatively low supercooling capacity and higher freezing injury temperatures as compared to the other three populations

TABLE 1. Relationship between supercooling temperature, freezing injury temperature and anatomical characteristics for five *Espeletia schultzei* populations

For epidermal and mesophyll cell size the mean \pm standard error for $n > 30$ cells/leaf obtained from two leaves of three different individuals are shown, RAWC = Relative apoplastic water content.

Population	Elevation (m)	Minimum average air temp. (°C)	Supercooled temp. (°C)	Injury temp. (°C)	RAWC (%)	Epidermal cell size (μm)	Mesophyll cell size (μm)
(1) Santo Domingo	2600	11.1	-5.3	-7.4	31.1	50.4 ± 12.9	31.9 ± 3.4
(2) Los Plantios	3100	3.6	-6.7	-8.8	16.0	35.1 ± 6.9	30.5 ± 3.7
(3) Mucubaji	3550	1.7	-9.2	-12.6	11.3	33.8 ± 8.5	26.5 ± 3.1
(4) Trasandina	3800	—	-10.3	-13.0	7.3	30.4 ± 7.1	26.9 ± 3.5
(5) Piedras Blancas	4200	-0.2	-13.2	-13.8	3.3	28.6 ± 6.9	20.7 ± 2.3

found above the limit of night frosts (Figs 1, 3). Based on these differences in freezing resistance capacity the populations may be grouped into two types: Santo Domingo (2 600 m) and Los Plantios (3 100 m) as type 1; and Mucubaji (3 550 m), Trasadina (3 800 m) and Piedras Blancas (4 200 m) as type 2. It is likely that there has been a stronger selection for differences in supercooling ability in type 2 populations which are frequently subjected to night-time freezing.

Tissue water status is known to affect supercooling capacity (Burke, Gusta, Quamme, Weiser, and Li, 1974; Timmis and Worrell, 1975). Furthermore, the number and magnitude of the exothermic events during freezing depend on the water content of the leaves (Salt and Kaku, 1967). In the genus *Espeletia*, Goldstein *et al.* (1985) have shown that water content influences the supercooling capacity of the leaves. The results of this study for a single species, *Espeletia schultzii*, support these previous findings. Supercooling capacity increases linearly with decreasing leaf water potentials, with plants from higher elevations being more sensitive to changes in Ψ_L than lower ones (Fig. 1). These patterns have an adaptive value since minimum air and plant temperatures occur during the dry season when leaf water potentials are lowest (Goldstein *et al.*, 1984).

The necessary qualifications for supercooling are not fully understood but they include: the absence of internal nucleators, barriers against external nucleators, the presence of antinucleators (substances which oppose the formation of nucleators), a relatively low moisture content, small cell size and little or no intercellular space for nucleation (Shearman, Olien, Marchetti, and Everson, 1973). We have observed that the water content and the leaf water potential influence the supercooling capacity of the leaves of *Espeletia schultzii*. We have also shown that cell size and both epidermis and mesophyll, decrease as elevation increases. And finally the probability of ice formation is reduced when the intercellular spaces are reduced since sites for ice nucleation and spreading will then be reduced (Levitt, 1980). This is another characteristic which changes along the gradient and together with the other qualifications suggest that anatomical differences may account for changes in the supercooling capacity of the leaves and show that selective pressures in *Espeletia schultzii* act towards a more efficient avoidance mechanism through supercooling as the plants reach higher altitudes.

George *et al.* (1974) have shown that in some temperate region species the appearance of the exotherm was strongly correlated with the minimum temperatures of their altitudinal and latitudinal distribution limit. This suggests the importance of supercooling as a cold resistance mechanism in the geographical and ecological distribution of these species. The supercooling characteristics observed in *Espeletia schultzii* show a clear example of its importance in the distribution of this species along the altitudinal gradient in the Andean Paramos.

ACKNOWLEDGEMENTS

We thank N. Holbrook for her comments on the manuscript and helpful suggestions. This research was supported by the Universidad de los Andes (CDCH) grant no. C-179-81, and by a CONICIT grant to the Postgrado de Ecología Tropical.

LITERATURE CITED

- BECK, E., SCHULZE, E., SENSER, M., and SCHEIBE, R., 1984. Equilibrium freezing of leaf water and extracellular ice formation in Afro-alpine 'giant rosette' plants. *Planta*, **162**, 276–82.
- , SENSER, M., SCHEIBE, R., STEIGER, H. M., and PONTGRANTZ, P., 1982. Frost avoidance and freezing tolerance in Afro-alpine giant rosette plants. *Plant, Cell and Environment*, **5**, 215–22.
- BURKE, M. J., GUSTA, L. V., QUAMME, H. A., WEISER, C. J., and LI, P. H., 1976. Freezing and injury in plants. *Annual Review of Plant Physiology*, **27**, 507–28.

- GEORGE, M. F., BURKE, M. J., PELLET, H. M., and JOHNSON, A. G., 1974. Low temperature exotherms and woody plant distribution. *HortScience*, **9**, 519–22.
- GOLDSTEIN, G., and MEINZER, F., 1983. Influence of insulating dead leaves and low temperatures on water balance in an Andean giant rosette species. *Plant, Cell and Environment*, **6**, 649–56.
- and MONASTERIO, M., 1984. The role of capacitance in the water balance of Andean giant rosette species. *Ibid.* **7**, 179–86.
- RADA, F., and AZOCAR, A., 1985. Cold hardiness and supercooling along an altitudinal gradient in Andean giant rosette species. *Oecologia*, **68**, 147–52.
- KAKU, S., and IWAYA, M., 1982. Low temperature exotherms in xylems of evergreen and deciduous broad-leaved trees in Japan with reference to freezing resistance and distribution range. In *Plant cold hardiness and freezing stress*. Eds P. H. Li and A. Sakai. Academic Press, New York. Pp. 227–39.
- LARCHER, W., 1971. Die Kalteresistenz von Obstbaumen und Ziergeholzen subtropischer Herkunft. *Oecologia plantarum*, **6**, 1–14.
- 1982. Typology of freezing phenomena among vascular plants and evolutionary trends in frost acclimation. In *Plant cold hardiness and freezing stress*. Eds P. H. Li and A. Sakai. Academic Press, New York. Pp. 417–26.
- LEVITT, J., 1980. *Responses of plants to environmental stresses. Volume 1. Chilling, freezing and high temperature stresses*. Academic Press, New York. 2nd edition.
- MEINZER, F., and GOLDSTEIN, G., 1984. Water and energy economy adaptations in Andean giant rosette plants. In *Evolutionary constraints on primary productivity: Adaptive strategies of energy capture in plants*. Eds T. Givnish and R. Robichaux. Cambridge University Press (in press).
- and RUNDEL, P., 1985. Morphological changes along an altitudinal gradient and their consequences for an Andean giant rosette plant. *Oecologia*, **65**, 278–83.
- MONASTERIO, M., and REYES, S., 1980. Diversidad ambiental y variación de la vegetación en los paramos de los Andes venezolanos. In *Estudios ecologicos de los Paramos Andinos*. Ed. M. Monasterio. Ediciones de la Universidad de los Andes, Merida, Venezuela. Pp. 47–91.
- QUAMME, H., STUSHNOFF, C., and WEISER, C., 1972. The relationship of exotherms to cold injury in apple stem tissues. *Journal of the American Society of Horticultural Science*, **97**, 608–13.
- RADA, F., 1983. Mecanismos de resistencia a temperaturas congelantes en *Espeletia spicata* y *Polylepis sericea*. Unpublished MS thesis. Universidad de los Andes, Merida, Venezuela.
- GOLDSTEIN, G., AZOCAR, A., and MEINZER, F., 1985. Freezing avoidance in Andean giant rosette plants. *Plant, Cell and Environment*, **8**, 501–7.
- RICHTER, H., DUHME, F., GLATZEL, G., HINCKLEY, T. M., and KARLIC, H., 1980. Some limitations and applications of the pressure–volume curve technique in ecophysiological research. In *Plants and their atmospheric environment*. Eds J. Grade, E. D. Ford and P. G. Jarvis. Blackwell, Oxford. Pp. 263–72.
- SALT, R. W., and KAKU, S., 1967. Ice nucleation and propagation in Spruce needles. *Canadian Journal of Botany*, **45**, 1335–46.
- SCHUBERT, C., and MEDINA, E., 1982. Evidence of quaternary glaciation in the Dominican Republic: Some implications for Caribbean paleoclimatology. *Paleogeography, Paleoclimatology, Paleocology*, **39**, 281–94.
- SHEARMAN, L. L., OLIEN, C. R., MARCHETTI, B. L., and EVERSON, E. H., 1973. Characterization of freezing inhibitors from winter wheat cultivars. *Crop Science*, **13**, 514–19.
- SMITH, A. P., 1974. Bud temperature in relation to nyctinastic leaf movement in an Andean giant rosette plant. *Biotropica*, **6**, 163–6.
- 1979. Function of dead leaves in *Espeletia schultzei* (Compositae), an Andean caulescent rosette species. *Ibid.* **11**, 43–7.
- SOKAL, R., and ROHLF, F., 1981. *Biometry*. 2nd Ed. WOH Freeman & Co.
- STEPONKUS, P., and LANPHEAR, F., 1967. Refinement of the triphenyl tetrazolium chloride method for determining cold injury. *Plant Physiology*, **42**, 1423–6.
- TIMMIS, R., and WORRALL, J., 1975. Environmental control of cold acclimation in Douglas Fir during germination, active growth and rest. *Canadian Journal of Forest Research*, **4**, 229–37.
- TYREE, M. T., and HAMMEL, H. T., 1972. The measurement of the turgor pressure on the water relations of plants by the pressure-bomb technique. *Journal of Experimental Botany*, **23**, 267–82.